A promiscuous prion: Efficient induction of [URE3] prion formation by heterologous prion domains

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ABSTRACT

The [URE3] and [PSI+] prions are the infectious amyloid forms of the *Saccharomyces cerevisiae* proteins Ure2p and Sup35p, respectively. Randomizing the order of the amino acids in the Ure2 and Sup35 prion domains while retaining amino acid composition does not block prion formation, indicating that amino acid composition, not primary sequence, is the predominant feature driving [URE3] and [PSI+] formation. Here we show that Ure2p promiscuously interacts with various compositionally similar proteins to influence [URE3] levels. Overexpression of scrambled Ure2p prion domains efficiently increases de novo formation of wild-type [URE3] in vivo. In vitro, amyloid aggregates of the scrambled prion domains efficiently seed wild-type Ure2p amyloid formation, suggesting that the wild-type and scrambled prion domains can directly interact to seed prion formation. To test whether interactions between Ure2p and naturally occurring yeast proteins could similarly affect [URE3] formation, we identified yeast proteins with domains that are compositionally similar to the Ure2p prion domain. Remarkably, all but one of these domains were also able to efficiently increase [URE3] formation. These results suggest that a wide variety of proteins could potentially affect [URE3] formation.
Amyloid fibril formation is associated with numerous human diseases, including Alzheimer disease, Type II diabetes and the transmissible spongiform encephalopathies. Yeast prions provide a powerful model system for examining amyloid fibril formation in vivo. [URE3] and [PSI+] are the prion forms of the *Saccharomyces cerevisiae* proteins Ure2p and Sup35p, respectively (WICKNER 1994). In both cases, prion formation is thought to result from conversion of native protein into an inactive amyloid form (GLOVER et al. 1997; KING et al. 1997; TAYLOR et al. 1999). Both proteins contain an N-terminal glutamine/asparagine (Q/N) rich prion domain and a C-terminal functional domain (LIEBMAN and DERKATCH 1999; MADDELEIN and WICKNER 1999; MASISON and WICKNER 1995; TER-AVANESYAN et al. 1994; TER-AVANESYAN et al. 1993). Sup35p contains an additional highly charged middle domain (M) that is not required either for prion formation or for normal protein function, but stabilizes [PSI+] aggregates (LIU et al. 2002).

Amyloid fibril formation is thought to occur through a seeded polymerization mechanism. In vitro, amyloid fibril formation from native proteins is generally characterized by a significant lag time, thought result from the slow rate of formation of amyloid nuclei; addition of a small amount of preformed amyloid aggregates (seeds) eliminates the lag time, resulting in rapid polymerization (GLOVER et al. 1997; SERIO et al. 2000; TAYLOR et al. 1999).

Despite considerable study, the mechanism by which amyloid seeds initially form is unclear. A variety of evidence suggests that at least some of the amyloid proteins involved in human disease can interact with unrelated amyloidogenic proteins, resulting in cross-seeding and modulation of toxicity. Injecting mice with amyloid-like fibrils
formed by a variety of short synthetic peptides promotes amyloid formation by amyloid protein A, a protein whose deposition is found in systemic AA amyloidosis (JOHAN et al. 1998). In yeast, \([PSI^+]\) and \([PIN^+]\), the prion form of the protein Rnq1p (DERKATCH et al. 2001; SONDHEIMER and LINDQUIST 2000), both promote the aggregation of and increase toxicity of expanded poly-glutamine tracts, like those seen in Huntington’s disease (DERKATCH et al. 2004; DUENNWALD et al. 2006; GOKHALE et al. 2005; MERRIIN et al. 2002; OSHEROVICH and WEISSMAN 2001); however, in Drosophila, \([PSI^+]\) aggregates reduce poly-glutamine toxicity (LI et al. 2007). These results demonstrate that interactions between heterologous amyloidogenic proteins can influence amyloid formation in vivo.

A variety of interactions have been observed among the yeast prions. Under normal cellular conditions, efficient formation, but not maintenance, of \([PSI^+]\) requires the presence of \([PIN^+]\) (DERKATCH et al. 2000). Overexpression of a variety of Q/N-rich proteins can effectively substitute for \([PIN^+]\), allowing \([PSI^+]\) formation in cells lacking \([PIN^+]\) (DERKATCH et al. 2001; OSHEROVICH and WEISSMAN 2001). Both in vitro and in vivo evidence suggest that the ability of \([PIN^+]\) to facilitate \([PSI^+]\) formation is the result of a direct interaction between Rnq1p aggregates and Sup35p (BARDILL and TRUE 2009; CHOE et al. 2009; DERKATCH et al. 2004). \([PIN^+]\) also increases the frequency of [URE3] formation, while \([PSI^+]\) inhibits [URE3] formation (BRADLEY et al. 2002; SCHWIMMER and MASISON 2002).

It is unclear whether the ability of Ure2p, Sup35p and Rnq1p to cross-react is an intrinsic feature of all similar amyloidogenic proteins, or whether it has specifically evolved to regulate prion formation. There is some debate as to whether yeast prion
formation is a beneficial phenomenon, allowing for regulation of the activity of the prion protein (TRUE et al. 2004; TRUE and LINDQUIST 2000), or a deleterious event analogous to human amyloid disease (NAKAYASHIKI et al. 2005). Either way, it is likely that interactions between the yeast prion proteins have specifically evolved, either to minimize the detrimental effects of amyloid formation or to regulate beneficial amyloid formation.

For both Ure2p and Sup35p, the amino acid composition of the prion domain is the predominant feature that drives prion formation. Scrambled versions of Ure2p and Sup35p (in which the order of the amino acids in the prion domain was randomized while maintaining amino acid composition) are able to form prions when expressed in yeast as the sole copy Ure2p or Sup35p (ROSS et al. 2004; ROSS et al. 2005). Here, to examine whether amino acid composition can similarly drive interactions between heterologous proteins, we tested whether the scrambled prion domains can interact with their wild-type counterparts to stimulate prion formation. We find that when overexpressed, scrambled Ure2 prion domains promote de novo prion formation by wild-type Ure2p, suggesting that the Ure2p prion domain can promiscuously interact with compositionally similar prion domains during prion formation. However, there are limits to this promiscuity; overexpression of wild-type or scrambled Sup35 prion domains does not increase [URE3] levels. Furthermore, we searched the yeast proteome for proteins with regions of high compositional similarity to Ure2p. Four of five of these proteins are able to efficiently stimulate [URE3] formation. We propose that this ability to promiscuously interact may have evolved as a mechanism to regulate Ure2p activity and/or prion formation.
MATERIALS AND METHODS

Strains and Media: Standard yeast media were as previously described (SHERMAN 1991). Galactose/raffinose dropout medium contained 2% galactose and 1% raffinose. In all experiments, yeast were grown at 30°C.

[URE3] induction by scrambled prion domains: Strain YER135 (MATa ura2 leu2 his3 trp1 URE2::HIS3 [PIN⁺], Σ1278b background) was transformed with either pH317 (EDSKEs and WICKNER 2000), a 2 μm, LEU2 plasmid carrying the GAL1 promoter, or with the previously described derivatives of pH317 in which the inducing prion domain was inserted under control of the GAL1 promoter (ROSS et al. 2004). Strains were grown for three days in galactose/raffinose dropout medium lacking leucine. Serial 10-fold dilutions were plated on SD+USA+Trp to select for [URE3] cells. Colonies were counted after five days and the plates were photographed after seven days. Frequencies of USA⁺ colony formation were determined as the mean of at least three independent experiments.

Stability, dominance, curability and cytoduction of [URE3]: To test for stability of the USA⁺ phenotype, USA⁺ colonies were resuspended in water in a 96-well microtiter plate and spotted onto YPAD plates. After 48 h, the cells from the YPAD plates were resuspended in water in a 96-well microtiter plate and spotted onto SD+USA+Leu+Trp to test for maintenance of the USA⁺ phenotype (this method is similar to replica plating, but transfers a more reproducible, lower density of cells, thereby providing cleaner results).
To test for dominance of the USA\(^+\) phenotype, USA\(^+\) colonies were spotted from the microtiter plates onto YPAD plates spread with a lawn of YER214 (\textit{MATa ura2 leu2 his3 ade2 URE2::HIS3}). These plates were grown for 24 h and replica plated to SD+Ura+Leu to select for diploids. The SD+Ura+Leu plates were grown for 48 h. Diploids were resuspended in water in a 96-well microtiter plate and spotted onto SD+USA+Leu to test diploids for [URE3].

To test curability, USA\(^+\) cells were resuspended in water in a 96-well microtiter plate and spotted onto YPAD plates and YPAD plus 5 mM guanidine. After 48 h, the cells from both plates were resuspended in water in a 96-well microtiter plate re-spotted onto the same medium (YPAD or YPAD plus 5 mM guanidine HCl). After another 48 h, cells were resuspended in water in a 96-well microtiter plate and spotted onto SD+USA+Leu+Trp to test for [URE3].

YER216 (\textit{MATa kar1 ura2 ade2 leu2 his3 \(\rho^0\)}) was used as a cytoduction recipient. \textit{kar1} reduces the efficiency of karyogamy during mating (Conde and Fink 1976), allowing for cell fusion and transfer of cytoplasmic material, without nuclear fusion. Donor (\(\rho^+\)) and recipient (\(\rho^0\)) cells were mixed in water and spotted onto YPAD. After incubation for 8 h at 30°C, cells were streaked onto medium selecting for recipient cells. Cytoductants were identified as \(\rho^+\) cells with the recipient’s nuclear genotype (Ridley et al. 1984).

[\textit{PSI}\(^+\)] generation: Yeast strains 780-1D/pJ533 (Song et al. 2005; from Dan Masison, National Institutes of Health) expressing wild-type \textit{SUP35}, and versions of 780-1D/pJ533 modified to express each of the scrambled versions of \textit{SUP35} (YER259, 289, 290, 292
and 293) were previously described (Ross et al. 2005). Strains were transformed with either pKT24 (from Kim Taylor, NABI, Rockville, MD), a 2 μm, TRP1 plasmid carrying the GAL1 promoter, or with a derivative of pKT24 in which the inducing prion domain was inserted under control of the GAL1 promoter (Ross et al. 2005). Strains were grown for 3 days in galactose/raffinose dropout medium lacking tryptophan. Serial 10-fold dilutions were spotted onto SC-ade medium to select for $[PSI^+]$ cells and grown for 5 days.

**Western blot analysis:** The prion domains of *URE2; URE2-21, -22, -23, -24, and -25; SUP35; and SUP35-21, -24, -25, -26 and -27*, as well as the fragments from *SAP30, GPR1, GIS1, PDC2* and *YLR278C*, were amplified by PCR from the respective inducing plasmids using primer EDR68 paired with EDR1057-1068, respectively (see Table S1 for oligonucleotides). These PCR products were then reamplified with EDR1055 and 1056. Together, these PCR reactions inserted a GGSGGSY spacer, Hemagglutinin (HA2) tag and stop codon at the carboxy terminus of each prion domain. PCR products were digested with *BamHI* and *XhoI* and inserted into *BamHI/XhoI* cut pH317 (Edskes and Wickner 2000). Ligation products were transformed into *Escherichia coli* and analyzed by DNA sequencing.

The resulting LEU2 plasmids were transformed into YER135. Cells were grown overnight in galactose/raffinose dropout medium lacking leucine, then diluted to OD$_{600} = 0.1$ and grown to OD$_{600} = 0.4-0.6$. Cells from 10 ml of culture were collected by centrifugation and then washed once with and resuspended in 25 mM Tris phosphate supplemented with 2 mM phenylmethanesulphonylfluoride (PMSF). Cells were lysed by
vortexing with glass beads (10 x 15 sec). Protein concentrations were determined by Bradford assay (Sigma). 5 micrograms protein was separated electrophoretically on SDS/12% PAGE gels and detected by western blot. Mouse monoclonal anti-HA antibody (Covance; HA.11) was used as the primary antibody and Alexa Fluor IR800 goat anti-mouse (Rockland) was used as the secondary antibody.

[URE3] loss: Four independent [URE3] isolates were tested. The genotype of YER2 (Figure 4A) is \textit{MAT}α \textit{ura2 leu2}, \textit{Σ}1278b background. YER223, 224 and 225 (Figure 4B, C and D, respectively) are [URE3] isolates of YER135. Each [URE3] isolate was transformed with either pH317 (EDSKES and WICKNER 2000) or a derivative of pH317 in which a prion domain was inserted under control of the \textit{GAL1} promoter. Strains were grown for 4 days in galactose/raffinose dropout medium lacking leucine. Strains were maintained in log phase by monitoring the culture’s OD$_{600}$ and diluting cultures 10- to 100-fold when the OD reached 0.2-0.6. At 24 h intervals, cells were plated for single colonies on YPAD. Single colonies were resuspended in water in a 96-well microtiter plate and spotted onto SD+USA+Trp+Leu to test for loss of [URE3]. For each strain/plasmid combination at each time point, a minimum of 20 colonies were tested. The fraction of cells that maintained the ability to grow on SD+USA+Trp+Leu is reported. Confidence intervals were calculated using the Adjusted Wald Method (AGRESTI and COULL 1998).

Colocalization studies. To cherry tag the wild-type and scrambled Ure2 prion domains, the prion domains were amplified by PCR from the respective inducing plasmids using
primer EDR68 paired with EDR1057-1061, respectively (see Table S1 for oligonucleotides). The mCherry (SHANER et al. 2004) ORF was amplified with EDR1187 and 1189. The product of the mCherry reaction was combined with each of the prion domain PCRs and reamplified with EDR68 and 1189. PCR products were digested with BamHI and XhoI and inserted into BamHI/XhoI cut pH317 under control of the GAL1 promoter. Ligation products were transformed into Escherichia coli and analyzed by DNA sequencing.

pH327, a CEN plasmid expressing Ure2-GFP from the URE2 promoter (EDSKES et al. 1999) was transformed into a [URE3] isolate of YER135. Each of the plasmids expressing cherry-tagged prion domains was transformed into this strain. Cells were grown for 12 h in galactose/raffinose dropout medium lacking leucine and tryptophan. Cells were visualized by confocal microscopy.

Protein expression and purification: Plasmids pER107, pER108 and pER111 expressing His6-tagged versions of the Ure2-21, -22 and -25 prion domains, respectively, are previously described (ROSS et al. 2004). Plasmid pER94 expresses His6-tagged full-length Ure2p (ROSS et al. 2004). Full-length Ure2p was expressed in Escherichia coli BL21 in 2xYT medium (1.6% Bacto Yeast Extract, 1% Bacto Tryptone and 0.5% sodium chloride, pH 7.0) containing 0.1 mg/ml ampicillin at 37 °C for 4 h after induction with 1 mM isopropyl β-D-thiogalactosidase at an A600 of ~1.0. After harvesting, cells were resuspended and sonicated in native lysis buffer (50 mM Tris-HCl, 300 mM NaCl, 5% glycerol, 0.02% NaN3, 10 mM imidazole), containing protease inhibitors (Complete
EDTA-free, Roche Applied Science). Insoluble material was removed by centrifugation (25 min at 15,000 x g).

Full-length Ure2p was recovered using an 11 ml Ni\textsuperscript{2+} HiTrap Chelating HP column (GE Heathcare). The protein was bound to the column in 50 mM Tris-HCl, 300 mM NaCl, 5% glycerol, 0.02% NaN\textsubscript{3}, 10 mM imidazole. Elution buffer was 50 mM Tris-HCl, 300 mM NaCl, 5% glycerol, 0.02% NaN\textsubscript{3}, 500 mM imidazole. Column was washed in three steps with a mixture of binding and elution buffer of 5%, 10% and 30% elution buffer, respectively. Protein was eluted in 100% elution buffer. Purified protein was diluted to 60 \(\mu\)M in 50 mM Tris-HCl, 0.2 M NaCl) and frozen at -70°C.

Prion domains were expressed in the same manner as full-length Ure2p and purified as previously described (BAXA et al. 2003). Purified prion domains were dialyzed into water to initiate fibril formation.

**In vitro fibril formation assay:** Amyloid fibril formation was monitored at 37°C by thioflavin T (ThT, Calbiochem) fluorescence using a Victor3 microplate reader (Perkin Elmer, Waltham, MA) with excitation and emission wavelengths of 460 and 490 nm, respectively. ThT solution was preincubated at 37°C for 1 h. Soluble full-length Ure2p was added to a concentration of 50 \(\mu\)M, with 1 mM ThT. High concentration seeding reactions (Figure 5A) contained 4% seed relative to full-length protein (mol/mol). Plates were sealed with sealing tape and shaken slowly with a 5 mm circular motion for 5 sec every min with emission measurements taken every 5 min. For unseeded reactions, fluorescence of a ThT blank was subtracted; for seeded reactions, the fluorescence signal from reactions containing only ThT and seed was subtracted. Low concentration seeding
reactions (Figure 5B) contained 0.4% (mol/mol) seed relative to full-length Ure2p. Additionally, for low concentration seeding reactions, a 5 mm glass bead was added to each well of the microtiter plate and between scans, plates were shaken continuously at 230 rpm at 37°C. Each sample was prepared in triplicate.

**Plasmids expressing fragments of yeast proteins:** The yeast proteome was scanned using a 45 amino acid window size, scoring every window based on the Euclidian distance of its amino acid composition from that of the Ure2p prion domain. For the top five scoring window, the window size was expanded to 89 amino acids in three steps. The 89 amino acid region of maximum Q/N content that contained the original 45 amino acid fragment was selected. If adjusting the boundaries while maintaining the 89 amino acid length could eliminate a proline residue without eliminating more than one Q/N, such adjustments were made. If possible without changing proline content, and without changing Q/N content by more than one, the boundaries were further adjusted to bring the net charge closer to ±1.

Selected regions were amplified by PCR (see Table S1 for oligonucleotides) from yeast strain YER135, installing a stop codon at the end of the selected region. PCR products were digested with BamHI and XhoI and inserted into BamHI/XhoI cut pH317 (EDSKES and WICKNER 2000). Ligation products were transformed into *Escherichia coli* and analyzed by DNA sequencing. The resulting LEU2, 2μm plasmids expressing fragments from *SAP30, PDC2, GIS1, GPR1* and *YLR278C* were named pER334, 336, 338, 340 and 342, respectively. The plasmid expressing full-length *SAP30* is pER538.
RESULTS

[URE3] induction by scrambled prion domains: The ability to utilize ureidosuccinate (USA) can be used to monitor [URE3] formation. In the presence of a good nitrogen source, Ure2p prevents uptake of USA, an intermediate in uracil biosynthesis. Loss of Ure2p function, either due to prion formation or a mutation in the URE2 gene, allows cells to take up USA. Transient overexpression of the Ure2p prion domain in [ure-o] cells, cells lacking the [URE3] prion, significantly increases the appearance of USA+ colonies (WICKNER et al. 1995). Surprisingly, we found that overexpression of each of the scrambled Ure2 prion domains also efficiently increased the frequency of USA+ colony formation (Table 1, Figure 1).

Because the USA+ phenotype results from loss of Ure2p activity, it can result either from a loss-of-function mutation in the URE2 gene or from [URE3] formation. To confirm that the increase in USA+ colony formation was a result of prion formation, we tested whether for each USA+ colony whether the USA+ phenotype was stable, dominant, curable and transmissible. For a significant fraction of the USA+ colonies induced by each of the scrambled prion domains, the USA+ phenotype was stable (Table 1; Figure 2A) and dominant (Table 1 and Figure 2B). Additionally, for all stable dominant USA+ colonies, the USA+ phenotype was curable by low concentrations of guanidine HCl (Figure 2C), which has been shown to cure [URE3] and [PSI+] (TUITE et al. 1981; WICKNER et al. 1995) by inhibiting the chaperone Hsp104p (FERREIRA et al. 2001; JUNG et al. 2002; JUNG and MASISON 2001). Finally, in all cases the USA+ phenotype was efficiently transmitted by cytoduction, a technique that allows for transmission of
cytoplasmic elements such as prions, but not of chromosomal elements (Conde and Fink 1976). Together, these data demonstrate that the increase in USA^+ colony formation upon scrambled prion domain overexpression is due to an increase in [URE3]-containing cells.

**Limits of prion promiscuity:** The wild-type and scrambled versions of Ure2p have identical amino acid compositions. We therefore tested whether less closely related prion proteins could similarly stimulate wild-type [URE3] formation. Specifically, we tested whether overexpression of wild-type or scrambled Sup35 prion domains could induce [URE3] formation. In fact, overexpression of both wild-type and scrambled Sup35 prion domains actually appeared to slightly suppress USA^+ colony formation by wild-type Ure2p (Table 1). This suggests that although Ure2p may interact with wild-type and scrambled Sup35 prion domains, such interactions are not able to productively nucleate [URE3] formation. That the wild-type Sup35 prion domain could not induce [URE3] formation was not surprising, as [PSI^+] is known to destabilize [URE3] aggregates (Schwimmer and Masison 2002). However, the scrambled Sup35 prion domains, although compositionally identical, have no primary sequence identity with the wild-type Sup35 prion domain. Therefore, these results highlight the critical role that amino acid composition plays in the induction of [URE3] by heterologous prion domains.

To confirm that differences in ability to induce [URE3] formation were not due to differences in efficiency of overexpression, expression of each of the wild-type and scrambled Sup35 and Ure2 prion domains was analyzed by western blot. Each prion domain was HA2-tagged and inserted into a plasmid under control of the GAL1 promoter.
Each of the HA-tagged prion domains stimulated [URE3] formation with similar efficiency to its untagged counterpart (data not shown). The expression levels of all of the prion domains exceptSUP35-25 were comparable to the wild-type URE2 prion domain (Figure 1B, 3A), demonstrating that the failure of the wild-type and scrambled Sup35 prion domains to induce [URE3] was not a result of inefficient overexpression.

Furthermore, each of the scrambled Sup35 prion domains was able to induce [PSI+] prion formation in strains expressing the full-length version of the same scrambled SUP35 (Figure 3B), although consistent with its poor expression (Figure 3A), prion formation by SUP35-25 was induced with the lowest efficiency. These results further confirm that their failure to induce [URE3] was not a result of inefficient overexpression. [PSI+] was detected by monitoring nonsense suppression of the mutant ade2-1 allele (Cox 1965). ade2-1 mutants are unable to grow without adenine and form red colonies in the presence of limiting adenine due to accumulation of a pigment derived from the substrate of Ade2p. In cells containing the weak nonsense suppressor tRNA SUQ5 (SUP16), [PSI+] cells are able to grow in the absence of adenine and form white or pink colonies when grown on media containing limiting adenine.

To test whether the ability to interact with compositionally identical prion domains was unique to Ure2p, we tested whether overexpression of heterologous prion domains could similarly increase [PSI+] colony formation. None of the scrambled Sup35 prion domains stimulated Ade+ colony formation in strains expressing wild-type SUP35 (Figure 3C). Similarly, overexpression of wild-type and scrambled Ure2 prion domains failed to increase Ade+ colony formation (data not shown).
[URE3] destabilization by scrambled prion domains: The observed ability of the scrambled prion domains to increase [URE3] populations could either be a result of direct cross-seeding or of an indirect mechanism such as titration of an inhibitor of [URE3] formation. Overexpression of the Ure2p prion domain fused to GFP results in loss of [URE3] (EDSKES et al. 1999). It was proposed that these fusion proteins bind to prion fibrils and “poison” their growth (EDSKES et al. 1999) or that overexpression results in aggregates that are too big to propagate (CRAPEAU et al. 2009). We hypothesized that if the scrambled prion domains can interact with wild-type Ure2p, they might similarly be able to destabilize [URE3] prions.

A plasmid overexpressing either wild-type or scrambled Ure2p prion domains from the GAL1 promoter was introduced into four strains carrying different [URE3] prion variants, each expressing a chromosomal copy of wild-type URE2. Each of these four [URE3] variants was isolated in a different manner – one by de novo [URE3] formation (YER2; Figure 4A), one by overexpression of the wild-type Ure2 prion domain (YER223; Figure 4B), one by overexpression of the Ure2-21 prion domain (YER224; Figure 4C) and one by overexpression of the Ure2-22 prion domain (YER225; Figure 4D). Cells were grown in galactose medium to induce expression from the GAL1 promoter; loss of the ability to utilize USA was used to monitor prion loss. Each of the scrambled prion domains destabilized wild-type [URE3] in all strains tested (Figure 4). The scrambled prion domains varied in their efficiencies of [URE3] destabilization, and none were as efficient as the wild-type prion domain, although in many cases the differences were not statistically significant (see Table S2 for raw data and confidence intervals). Curing by the scrambled prion domains was only modestly affected by the
manner in which the [URE3] prion was originally isolated; although both the Ure2-21 and Ure2-22 prion domains were most efficient at curing prions that had originally been formed by their overexpression (strains YER224 and 225, respectively), these differences were not statistically significant. Similar results were seen for prions induced by each of the other scrambled prion domains (data not shown). These results are consistent with a direct physical interaction between wild-type and scrambled Ure2p.

Scrambled Ure2p prion domains colocalize with wild-type Ure2p aggregates. Ure2-GFP fusions form foci in [URE3] cells, but not in [ure-o] cells (EDSKES et al. 1999). We expressed Ure2-GFP fusions in a [URE3] cell, and then transiently overexpressed mCherry (SHANER et al. 2004) tagged wild-type or scrambled Ure2 prion domain. As expected, the wild-type prion domain consistently colocalized with Ure2-GFP foci (Figure 5A). The results for the cherry-tagged scrambled Ure2 prion domains were more variable. In many cells containing wild-type Ure2-GFP foci, the scrambled Ure2 prion domains appeared diffuse, or in foci that were distinct from the GFP foci [Figure 5B shows one such example for Ure2-21; similar results were seen for the other scrambled prion domains (data not shown)]. However, in a subset of cells, the scrambled prion domains were co-localized with the wild-type Ure2-GFP foci (Figure 5B). Although the frequency of this colocalization varied among the prion variants, in all cases some colocalization was observed (data not shown). This is consistent with a direct interaction between wild-type Ure2p and scrambled Ure2 prion domains, but suggests that this interaction is transient and/or less efficient that the interaction between Ure2 and the wild-type Ure2 prion domain.
**Scrambled Ure2p prion domains directly seed Ure2p amyloid formation:** The rate limiting step for in vitro amyloid formation is the initial formation of amyloid nuclei. Once nuclei are formed, polymerization proceeds rapidly. Therefore, in vitro Ure2p amyloid formation is characterized by a significant lag phase, followed by rapid polymerization; addition of a small amount of preformed Ure2p amyloid fibrils eliminates the lag phase (Taylor et al. 1999; Figure 6). To determine whether the increase in wild-type [URE3] formation upon overexpression of the scrambled Ure2p prion domains is a result of direct cross seeding, we examined in vitro whether amyloid fibrils of the scrambled prion domains could similarly seed amyloid formation by wild-type Ure2p. Three of the scrambled Ure2p prion domains were purified under denaturing conditions and dialyzed into water to initiate amyloid formation. A small amount of these fibers was added to purified full-length Ure2p. At 4% concentration relative to the full-length Ure2p (mol/mol), each scrambled prion domain was able to completely eliminate the lag time for Ure2p amyloid formation (Figure 6A). At much lower concentrations (0.4%), each scrambled prion domain was able to shorten the lag time with efficiencies comparable to that of wild-type URE2 seed (Figure 6B). These data are consistent with direct seeding of wild-type Ure2p amyloid formation by the scrambled prion domains.

**Induction of [URE3] formation by fragments of yeast proteins:** The yeast proteome contains ~100 proteins with regions of similar Q/N content to Ure2p (Michelitsch and Weissman 2000). We performed a search to identify the yeast proteins with regions that
are most compositionally similar to that of the Ure2p prion domain. Because ~40-50 amino acids is the minimum length required for efficient [URE3] induction (Ross et al. 2005), we scanned the yeast proteome using a 45 amino acid window size, scoring each window based on the Euclidean distance of its amino acid composition from that of the Ure2p prion domain (amino acids 1-89).

The fragments closest in amino acid composition to the Ure2p prion domain were from SAP30, PDC2, GIS1, GPR1 and YLR278C (Table 2). Although 40-50 amino acids of the Ure2p prion domain are sufficient for prion induction, in general longer prion domain fragments induce more efficiently (Ross et al. 2005). Therefore, for each 45 amino acid fragment identified by the search, an 89 amino acid segment containing the 45 amino acid fragment was selected for testing (Figure 7A). The 89 amino acid fragment was chosen to 1) maximize Q/N content, 2) minimize proline content, as prolines have been shown to disrupt β-sheet formation, and 3) when possible, have the fragments net charge be ±1, as these net charges have been shown to be ideal for amyloid formation by small peptides (Lopez de la Paz et al. 2002). These fragments were inserted into plasmids under control of the GAL1 promoter and used for induction experiments. Of the five fragments, only that of PDC2 failed to efficiently induce USA⁺ colony formation (Table 2; Figure 7B). The failure of PDC2 was not due to poor expression; all of the fragments except that of GIS1 showed comparable expression levels (Figure 7C). Remarkably, three of the fragments, from SAP30, GPR1 and YLR278C, increased USA⁺ colony formation by greater than 30-fold; by contrast, overexpression of the wild-type prion domain only increased USA⁺ colony formation by approximately 20-fold. For many of the USA⁺ colonies induced by overexpression of the SAP30, GIS1,
and YLR278C fragments, the USA\(^+\) phenotype was stable and dominant (Table 2), consistent with these fragments causing [URE3] formation. All stable dominant USA\(^+\) colonies were curable by guanidine HCl (data not shown). Additionally, for all dominant, stable USA\(^+\) colonies tested (at least two for each induction), the USA\(^+\) phenotype was transmissible by cytoduction (data not shown). Together, these data demonstrate that the fragments from SAP30, GIS1, GPR1 and YLR278C are each able to increase the frequency of [URE3].

**DISCUSSION**

Because the events that initiate amyloid formation in human disease are not well understood, identifying the factors that contribute to amyloid formation is critical for understanding amyloid disease. Experiments from a variety of systems indicate that amyloidogenic proteins can cross-react to seed amyloid formation. However, heterologous cross-seeding is generally thought of highly inefficient; for example, although Rnq1p aggregates can cross-seed Sup35p amyloid formation, Rnq1p aggregates are at least fifty-fold less efficient than Sup35p aggregates at seeding aggregation of soluble Sup35p in vitro (DERKATCH *et al.* 2004). The significant finding here is that heterologous prion domains can promote [URE3] formation at efficiencies rivaling that of the homologous prion domain.

The increase in [URE3]-containing cells upon overexpression of scrambled prion domains could result from either direct cross-seeding or an indirect mechanism. For example, prion domain overexpression could upregulate expression of prion-promoting proteins. Previous microarray analysis indicates that [URE3] formation, even in the
presence of prion domain overexpression, does not cause any significant gene expression changes beyond those attributable to nitrogen derepression (ROSS and WICKNER 2004), arguing against a transcriptional change; nevertheless, such changes could occur post-transcriptionally or could be below the threshold of detection by microarray. Prion domain overexpression could also titrate away an inhibitor of prion formation, thereby promoting prion formation by the full-length protein. However, the ability of the scrambled prion domains to seed wild-type Ure2p amyloid formation in vitro in the absence of any additional proteins, the colocalization of scrambled prion domains with wild-type [URE3] aggregates and the ability of scrambled prion domains to destabilize existing [URE3] aggregates all argue against these indirect mechanisms and in favor of a direct cross-seeding mechanism.

Structural studies of amyloid fibrils of the yeast prion proteins provide some insight into how amyloid formation can be seeded by heterologous prion domains. NMR studies indicate that Sup35p, Ure2p and Rnq1p amyloid fibrils, as well as those of scrambled versions of Ure2p and Sup35p, are composed of in-register parallel β-sheets (BAXA et al. 2007; SHEWMAKER et al. 2008; SHEWMAKER et al. 2006; WICKNER et al. 2008), although alternative models for both Ure2p and Sup35p amyloid fibrils have been proposed (BOUSSET et al. 2002; KRISHNAN and LINDQUIST 2005). The prion domains of Sup35p, Ure2p and Rnq1p are all Q/N-rich, and stacking of Q/N residues to form polar zippers has been proposed to stabilize amyloid fibrils (PERUTZ et al. 2002; TSAI et al. 2005). Therefore, it is likely that the Q/N residues allow for interactions between Sup35p, Ure2p, and Rnq1p, although it is interesting that [PIN+] can also stimulate amyloid formation in vivo by the non-Q/N-rich Het-s prion domain from Podospora...
anserina (TANEJA et al. 2007). It has been proposed that the ends of growing amyloid fibrils may act as an imperfect template for heterologous prion proteins, allowing the heterologous prion protein to add to the fiber end (WICKNER et al. 2008).

This idea explains both the ability of \([PIN^+]\) to seed amyloid formation by Sup35p and other amyloidogenic proteins (DERKATCH et al. 2004), and the relative inefficiency of this cross-seeding. In vivo, even in the presence of \([PIN^+]\), significant \([PSI^+]\) prion formation requires overexpression of the Sup35p prion domain. The rarity with which \([PIN^+]\) seeds \([PSI^+]\) formation in vivo presumably reflects the fact that fibers of Rnq1p are imperfect templates for Sup35p amyloid formation.

Therefore, it is quite surprising that scrambled versions of Ure2p can seed wild-type Ure2p amyloid formation almost as efficiently as wild-type Ure2p self-seeds. The seeding of \([URE3]\) formation by scrambled prion domains was significantly more efficient than any previously observed cross-seeding among yeast prions, both in vitro and in vivo, reflecting the uniquely promiscuous nature of Ure2p. Furthermore, these results are in sharp contrast to mammalian prion systems, in which changes of a few amino acids can create a species barrier, preventing prion transmission (reviewed in COLLINGE and CLARKE 2007).

Although Ure2p appears to have a unique ability to efficiently cross-react with scrambled Ure2p prion domains, there are clearly limits to its promiscuity. That wild-type Sup35 prion domain could not cross-seed \([URE3]\) formation was not surprising, as the Ure2p and Sup35p have co-evolved, and therefore may have specifically evolved to minimize their interaction. However, all of the scrambled Sup35p prion domains efficiently aggregate, yet all repressed formation of \([URE3]\) colonies. This suggests that
amino acid composition may be critical for determining whether Ure2p can productively interact with heterologous proteins to stimulate [URE3] formation. Although both the Ure2 and scrambled Sup35 prion domains are Q/N-rich (48.3% and 45.6%, respectively), they are only 46.9% compositionally identical (Table S3). Interestingly, the Ure2 prion domain is far more N-rich, while Sup35 is more Q-rich, raising the possibility that interactions between asparagines (but not interactions between asparagines and glutamines) may be involved in heterologous induction.

The failure of wild-type [PSI+] formation to be seeded by scrambled versions of Sup35p demonstrates that similar amino acid composition is not sufficient for efficient cross-seeding between heterologous Q/N-rich proteins. Clearly there must be some unique feature of Ure2p (either of the prion domain or the C-terminus) that allows it to be cross-seeded with such remarkable efficiency. Therefore, further studies to determine the basis for Ure2p’s promiscuity will be critical for determining whether similar heterologous cross-seeding events may be involved in disease-related amyloid formation.

The demonstration that fragments of other yeast proteins were also able to induce [URE3] formation raises the possibility that Ure2p’s promiscuity could be physiologically relevant. It is important to note that the identified domains may be less accessible for interaction within the context of their respective full-length proteins. However, the ability of all but one of these fragments to induce [URE3] formation indicates that the requirements for heterologous cross-seeding are quite broad. Although these fragments were identified as having similar composition to the Ure2p prion domain, the search algorithm was quite simple. The fragments have significant compositional deviations from that of the Ure2 prion domain, with 66.2-79.7% compositional identity.
(Table S3 for detailed amino acids compositions). We made no effort to bias the algorithm in favor of fragments with similar Q/N content to the Ure2 prion domain or against fragments that have residues such as prolines that are known to inhibit β-sheet formation. Consequently, these fragments had from 25 to 56 Q/N residues and from one to four prolines; by contrast, the Ure2 prion domain has 43 Q/N residues and no prolines. Given the high success rate of such a simple search algorithm, it is likely that at least some of the many other Q/N-rich proteins in yeast would similarly be able to induce [URE3] formation.

When the identified domains of GPR1, GIS1, SAP30, YLR278C and PDC2 were inserted in the place of the Ure2 prion domain, none of the domains appeared able to form stable prions (data not shown). However, it is still possible that some of these domains may form prions in their native context, or that they may form aggregates that are not stably propagated, but are nonetheless able to affect [URE3] formation or stability. Therefore, further study will be required to determine whether the respective full-length proteins can influence [URE3] formation.

Three of the identified domains (GPR1, SAP30, and PDC2) were recently identified in a separate bioinformatics search for potential prion domains (ALBERTI et al. 2009). Intriguingly, fragments from GPR1 and SAP30 were shown to form SDS-resistant aggregates when overexpressed and visible foci when fused to GFP; however, neither efficiently formed amyloid fibrils in vitro. By contrast, PDC2, which was the only fragment identified by our search that failed to induce [URE3], failed to form both foci and SDS-resistant aggregates upon overexpression (ALBERTI et al. 2009). These results
suggest that $PDC2$ may fail to induce $[URE3]$ formation simply because it has a lower propensity to aggregate in vivo.

The function, if any, of Ure2p’s unique promiscuity is unclear. It has been proposed that yeast prions may serve a beneficial function in cells (TRUE et al. 2004; TRUE and LINDQUIST 2000), although the failure to identify $[URE3]$ or $[PSI^+]$ in any wild yeast strains argues that such beneficial prion formation is at most a rare event (NAKAYASHIKI et al. 2005). Over the past year, the list of yeast prion proteins has rapidly grown, and now includes Mot3p (ALBERTI et al. 2009), Cyc8p (PATEL et al. 2009), Swi1p (DU et al. 2008), Mca1p (NEMECEK et al. 2009), Rnq1p (DERKATCH et al. 2001; SONDHEIMER and LINDQUIST 2000), Ure2p (WICKNER 1994) and Sup35p (WICKNER 1994). Ure2p’s promiscuity, combined with the growing list of yeast prions, raises the intriguing possibility that a complex network of prion protein interactions may affect $[URE3]$ formation and propagation.

The theory of beneficial prions states that cells normally exist in a non-prion state but are constantly sampling the prion state through spontaneous prion formation. If the prion state confers a selective advantage, prion-containing cells take over the population. Because $[URE3]$ cells grow slower than cells lacking $[URE3]$ under optimal growth conditions (WICKNER 1994), it would be disadvantageous for cells to form $[URE3]$ at a high frequency when growing under such ideal conditions. However, in the wild, yeast presumably frequently grow under non-ideal conditions. Therefore, it might be beneficial for yeast to develop mechanisms to suppress prion formation under ideal growth conditions and select for it during times of cellular stress; a cell that is about to die has little to lose by sampling the prion state. A variety of cellular stresses, many of
which disrupt protein folding, have been shown to increase the frequency of prion formation (CHERNOFF 2007; TYEDMERS et al. 2008). Perhaps the promiscuity of the Ure2p prion domain has evolved as a mechanism to increase prion formation in response to cellular stress. Because of Ure2p’s ability to promiscuously interact, aggregation of other Q/N-rich proteins as a result of cellular stress might increase [URE3] formation.

Alternatively, the promiscuity of the Ure2 prion domain may reflect a normal function of the protein. The prion domain is intrinsically disordered. For many proteins, regions of intrinsic disorder are used to recognize multiple binding targets, with the disordered domain adopting different structures upon binding to each target (HANSEN et al. 2006). Although it is unclear exactly what function the prion domain serves, when the prion domain is removed, Ure2p is less active and shows reduced ability to bind to Gzf3p, a component of the nitrogen regulation system (SHEWMAKER et al. 2007). The prion domain may have evolved to promiscuously interact with multiple targets. Thus, the ability to self-interact to form prion fibers and the ability of the prion formation to be seeded by heterologous proteins may simply be an unfortunate byproduct of this promiscuity.

Further experiments are needed to determine the function, if any, of Ure2p’s ability to interact with other Q/N-rich proteins during prion formation. However, our results clearly show that [URE3] formation can be modulated with remarkable efficiency by a variety of unrelated peptides. This demonstration that heterologous seeding can be quite efficient raises the important question of whether any human amyloidogenic proteins are similarly promiscuous.
Acknowledgments

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REFERENCES


Figure Legends:

FIGURE 1.—Induction of USA\(^+\) colonies by scrambled Ure2p prion domains. (A) Yeast strain YER135 expressing wild-type \textit{URE2} was transformed with either a plasmid containing the \textit{GAL1} promoter (vector) or expressing the indicated prion domain from the \textit{GAL1} promoter. Yeast were grown in galactose/raffinose medium for 3 days. \(5 \times 10^5\) cells were then plated onto USA medium to select for prion-containing cells. Cells were counted after 5 days and photographed after 7. (B) Western blots of wild-type and scrambled Ure2 prion domains. Plasmids containing the \textit{GAL1} promoter (vector) or HA-tagged prion domains expressed from the \textit{GAL1} promoter were introduced into yeast strain YER135. Cells were grown in galactose medium and harvested in log phase. Cell lysates were analyzed by western blot.

FIGURE 2.—Scrambled Ure2 prion domains induce stable, dominant and curable USA\(^+\) colonies. (A) Stability of the USA\(^+\) phenotype. USA\(^+\) colonies isolated upon overexpression of the wild-type Ure2 prion domain, scrambled prion domains (\textit{URE2-21, -22, -23, -24} and -25) or without overexpression (vector) were spotted onto YPAD and grown for 48h. Cells were then transferred to medium with uracil (right) or USA (left) to test for the ability to utilize USA. Shown are representative results, with 6 independent isolates induced each prion domain. (B) Dominance of the USA\(^+\) phenotype. USA\(^+\) colonies were spotted onto a lawn of [ure-o] cells of the opposite mating type and grown for 24 h. Cells were replica plated to select for diploids and grown for 48 h. Diploids were spotted onto medium with uracil (right) or USA (left) to test for the ability to utilize USA. Shown are representative results, from the same USA\(^+\) isolates as in panel A. (C)
Curability of the USA⁺ phenotype. USA⁺ colonies were spotted onto YPAD supplemented with 5 mM guanidine HCl and grown for 48 h. Cells were then spotted at low density onto fresh YPAD medium supplemented with 5 mM guanidine HCl and grown for an additional 48 h. Cells were then transferred to medium with uracil (right) or USA (left) to test for the ability to utilize USA. Shown are representative results, from the same USA⁺ isolates as in panel A.

FIGURE 3.–[PSI⁺] formation is not induced by scrambled Sup35p prion domains. (A) Western blots of wild-type and scrambled Sup35 prion domains. Plasmids containing the GAL1 promoter (vector) or HA-tagged prion domains expressed from the GAL1 promoter were introduced into yeast strain YER135. Cells were grown in galactose medium and harvested in log phase. Cell lysates were analyzed by western blot. (B) Induction of prion formation by scrambled Sup35s by homologous prion domains. Strains expressing the indicated full-length scrambled SUP35 as the sole copy of SUP35 were transformed with either a plasmid pKT24 containing the GAL1 promoter (-) or a derivative of pKT24 expressing the same scrambled Sup35 prion domain from the GAL1 promoter (+). Strains were grown in galactose/raffinose dropout medium, and then serial ten-fold dilutions were spotted onto SC-ade medium to select for [PSI⁺] cells. (C) Yeast strain 780-1D/pJ533 expressing full-length wild-type SUP35 was transformed with pKT24 (vector) or pKT24 modified to express each of the scrambled Sup35 prion domains. Strains were grown in galactose/raffinose dropout medium, and then spotted onto SC-ade medium to select for [PSI⁺] cells.
FIGURE 4.–Scrambled prion domains destabilize wild-type [URE3]. Plasmids containing the GAL1 promoter (vector) or expressing wild-type or scrambled Ure2 prion domain from the GAL1 promoter were introduced into four different [URE3] strain expressing wild-type URE2 from the URE2 genomic locus. Cells were grown in galactose/raffinose dropout medium for varying lengths of time and then plated onto YPAD. Individual colonies from YPAD were tested for loss of [URE3]. At least 20 colonies were tested at each time point. (A) Prion loss in yeast strain YER2. (B) Prion loss in yeast strain YER223. (C) Prion loss in yeast strain YER224. (D) Prion loss in yeast strain YER225.

FIGURE 5.–Colocalization of prion domains with wild-type [URE3] aggregates. [URE3] cells expressing Ure2-GFP were transformed with plasmids expressing the mCherry labeled prion-forming domain (PFD) of wild-type Ure2 (A) or Ure2-21 (B). For the Ure2-21 PFD, cells with (columns 2 and 3) and without (column 1) colocalization are shown. DIC, differential interference contrast.

FIGURE 6.–Scrambled prion domain aggregates seed wild-type Ure2p amyloid formation in vitro. Polymerization of Ure2p in a microplate was monitored by thioflavin T fluorescence with and without addition of preformed wild-type URE2 or scrambled prion domain seed. A) 4% seed relative to full-length protein (mol/mol) was added and plates were shaken for 5 sec every min with emission measurements taken every 5 min. B) 0.4% (mol/mol) seed relative to full-length Ure2p was added. To accelerate amyloid
fibril formation, plates were shaken continuously at 230 rpm at 37°C except during reading of fluorescence, resulting in a shorter lag time for amyloid formation.

FIGURE 7.—Induction of [URE3] by compositionally similar domains. (A) Amino acid sequences of the tested domains. The locations of the segments within the respective protein sequences is indicated. (B) Yeast strain YER135 expressing wild-type URE2 was transformed with either a plasmid containing the GAL1 promoter (vector) or expressing the indicated prion domain from the GAL1 promoter. Yeast were grown in galactose/raffinose medium for 3 days. 5x10^5 cells were then plated onto USA medium to select for prion-containing cells. Cells were counted after 5 days and photographed after 7. (C) Western blots of the compositionally similar fragments. Plasmids containing the GAL1 promoter (vector) or HA-tagged prion-like domains expressed from the GAL1 promoter were introduced into yeast strain YER135. Cells were grown in galactose medium and harvested in log phase. Cell lysates were analyzed by western blot.
TABLE 1
Induction of wild-type [URE3] by heterologous prion domains

<table>
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<tr>
<th>Overexpressed prion domain&lt;sup&gt;a&lt;/sup&gt;</th>
<th>USA&lt;sup&gt;+&lt;/sup&gt; colonies/10&lt;sup&gt;6&lt;/sup&gt; cells&lt;sup&gt;b&lt;/sup&gt;</th>
<th>USA&lt;sup&gt;+&lt;/sup&gt; stability&lt;sup&gt;c&lt;/sup&gt;</th>
<th>USA&lt;sup&gt;+&lt;/sup&gt; dominance&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Cytoduction&lt;sup&gt;e&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>Vector</td>
<td>1.3 ± 0.3</td>
<td>7/20</td>
<td>4/7</td>
<td>20/20</td>
</tr>
<tr>
<td>Wild-type&lt;sup&gt;f&lt;/sup&gt;</td>
<td>23 ± 3</td>
<td>12/20</td>
<td>8/12</td>
<td>18/20</td>
</tr>
<tr>
<td>URE2-21</td>
<td>17 ± 3</td>
<td>12/20</td>
<td>7/12</td>
<td>20/20</td>
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<tr>
<td>URE2-22</td>
<td>10 ± 5</td>
<td>18/20</td>
<td>17/18</td>
<td>20/20</td>
</tr>
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<td>URE2-23</td>
<td>130 ± 12</td>
<td>14/20</td>
<td>7/14</td>
<td>17/20</td>
</tr>
<tr>
<td>URE2-24</td>
<td>18 ± 1</td>
<td>15/20</td>
<td>7/15</td>
<td>14/20</td>
</tr>
<tr>
<td>URE2-25</td>
<td>27 ± 6</td>
<td>19/20</td>
<td>18/19</td>
<td>20/20</td>
</tr>
<tr>
<td>SUP35</td>
<td>&lt;1.0</td>
<td>5/20</td>
<td>1/5</td>
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<td>3/7</td>
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<td>6/20</td>
<td>5/6</td>
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<sup>a</sup> Yeast strain YER135 was transformed with either a plasmid containing the GAL1 promoter (pH317, vector) or pH317 modified to express the indicated prion domain from the GAL1 promoter.

<sup>b</sup> Yeast were grown in galactose/raffinose medium for 3 days and plated onto USA medium to select for prion-containing cells. Data are the number of USA<sup>+</sup> colonies per 10<sup>6</sup> cells and represent the mean of three independent experiments. Standard errors are indicated.

<sup>c</sup> That is, the fraction of USA<sup>+</sup> colonies that remained USA<sup>+</sup> after 48 h of growth on YPAD. N.D. means not determined.
That is, the fraction of stable USA⁺ colonies whose USA⁺ phenotype was dominant when mated with [ure-o] cells carrying a chromosomal copy of wild-type *URE2*.

Two stable dominant USA⁺ isolates were used as cytoduction donors. The [ure-o] recipient strain carried a wild-type chromosomal copy of *URE2*. The numbers indicated the fraction of cytoductants that were USA⁺.

Efficient induction by the wild-type prion domains has previously been reported (MASISON and WICKNER 1995), but is included here as a positive control.
**[URE3] induction by yeast protein fragments**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Proposed function</th>
<th>Region Identified</th>
<th>Fragment Tested</th>
<th>USA⁺ colonies/10⁶ cells ± SEM</th>
<th>USA⁺ Stability</th>
<th>USA⁺ Dominance</th>
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<td>vector</td>
<td>none</td>
<td>none</td>
<td>1.3 ± 0.6</td>
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<tr>
<td><strong>URE2</strong></td>
<td>1-89</td>
<td>24 ± 3</td>
<td>16/20</td>
<td>11/16</td>
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<td><strong>SAP30</strong></td>
<td>Histone deacetylase</td>
<td>40-84</td>
<td>18-106</td>
<td>19/20</td>
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<tr>
<td><strong>PDC2</strong></td>
<td>Transcription regulator</td>
<td>535-579</td>
<td>511-599</td>
<td>13/20</td>
<td>10/13</td>
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<td><strong>GIS1</strong></td>
<td>Histone demethylase</td>
<td>775-819</td>
<td>752-840</td>
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<td><strong>GPR1</strong></td>
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<td><strong>YLR278C</strong></td>
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<td>886-930</td>
<td>886-974</td>
<td>19/20</td>
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* The yeast proteome was searched, using a 45 amino acid window size, to identify the proteins that contain regions that are most compositionally similar to the Ure2p prion domain. The five proteins containing the highest scoring regions and the proposed molecular functions of the proteins are indicated. For each protein, an 89 amino acid fragment was chosen that contained the identified region. This fragment was inserted into plasmid pH317 under control of the *GAL1* promoter and transformed into yeast strain YER135. USA⁺ colony formation, USA⁺ stability and USA⁺ dominance were tested as in Table 1.
Figure 1
Figure 2
Figure 3

A

wt Ure2
Vector
wt Sup35
Sup35-21
Sup35-24
Sup35-25
Sup35-26
Sup35-27

B

- +
Sup35-21
Sup35-24
Sup35-25
Sup35-26
Sup35-27

C

Vector
wt Sup35
Sup35-21
Sup35-24
Sup35-25
Sup35-26
Sup35-27
Figure 4
Figure 5
Figure 6
Figure 7

A

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<td>G81 (752-840)</td>
<td>KRHNILNHGMDNSNLSCNNPDYETCMQQGQNKTEILNNHMNNNNNDNYTP 5NNNNNGQQQASSFEPHEYNMNNMNNNNTYICEQNGQFSSG</td>
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<td>GPR1 (499-587)</td>
<td>DHHNNDMHHHNNNNNNNNNNNNNNNNNNNNNNNNNNNNN</td>
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<td>YLR278C (886-974)</td>
<td>YLR278C QKELKINNVNONGMTQDSVREHNHNNNNNNNNNN</td>
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B

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